La Diphotérine7 solution de lavage efficace: Documents et études de références

[Diphoterine7, an efficacious lavage solution: Reference documents and studies]

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DIPHOTERINE ACID LAVAGE RESIDUE
EVALUATION OF OCULAR IRRITATION
IN THE RABBIT

Adressee

Society Prevor
Mrs. D. Jahan
Moulin de Verville
95760 Valmondois
France

Date: 07/10/90

Study No. 6463 TAL

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[*Translators note: page numbers in the TABLE OF CONTENTS refer to those in the original French document]

CERTIFICATION OF THE STUDY DIRECTOR

This study has been carried out in conformity with the protocol established with Society Prevor, agreement of September 21, 1984 published in the Journal Officiel de la Républic Français (J.O.R.F. of October 21, 1984) [Official Journal of the French Republic] and according to the Good Laboratory Practice rules (France, Ministere del la Santé, May 31, 1983) [France, Ministry of Health].

I certify that this report is a true and accurate description of the procedures utilized and the results obtained while conducting this study.

This study was done at the Centre International de Toxicologie (C.I.T.) [International Toxicology Center] at Miserey, 27005 Evereux, France.

Toxicology

[Signature]

J. Clouzeau
Biologist
Head of the Local Tolerance
and Acute Toxicity Service

OTHER SCIENTIFIC PARTICIPANTS IN THIS STUDY

[Signature]

Pharmacy
Product Preparation

M.H. Read Date: 07/10/90
Pharmacist Biologist

Study No. 6463 TAL

ACCOUNT OF THE QUALITY ASSURANCE UNIT

The protocol and the report were inspected by the Quality Assurance Unit of the International Toxicology Center on the following dates:

Inspection	Inspection Date	Date of Inspection Report
Protocol	05/16/90	05/16/90
Report (1st Draft)	07/05/90	07/06/90
Report (final)	07/10/90	07/10/90

Studies (of the same type) were routinely inspected on the following dates:

Treatment/Product	06/05/90	06/05/90
Data Recording	06/05/90	06/05/90
Records	06/22/90	06/22/90
Product/Preparation	06/11/90	06/11/90

Inspections were carried out in accordance with the standard operating procedures of C.I.T. and Good Laboratory Practices (France, Ministere de la Santé, May 31, 1983) [France, Ministry of Health].

[Signature]

M. Labiche Date: 07/10/90
Head of the Quality
Assurance Unit and
Scientific Archives

Study No. 6463 TAL

SUMMARY

At the request of Society Prevor, ocular irritation which might be produced by the product **DIPHOTERINE7 ACID LAVAGE RESIDUE** was evaluated in the rabbit in conformity with the agreement of September 21, 1984 published in the Journal Officiel de la République Française [Official Journal of the French Republic] and according to Good Laboratory Practice rules (France, Ministere de la Santé, May 31, 1983) [France, Ministry of Health].

Materials and Methods

A single dose of 0.1 mL of the product was placed in the conjunctival sac of the left eye of 6 New Zealand white rabbits. No eye rinsing was done after application of the product. The ocular reaction was observed 1 hour after application, and then on days 2, 3, 4, 5, and 8.

Results

No ocular reaction was observed in these animals.

Conclusion

Under our experimental conditions and using the rating scale developed by Kay and Calandra, the product **DIPHOTERINE7 ACID LAVAGE RESIDUE** is considered to be **non-irritating** by the ocular route in rabbits.

1. MATERIALS AND METHODS

1.1 PRODUCT

The product **DIPHOTERINE7 ACID LAVAGE RESIDUE** furnished by Society Prevor, Moulin de Verville, 95760 Valmontois [sic], France was in the form of a colorless liquid.

The product was packaged in one glass bottle and was received at C.I.T. on 05/21/90 with the label AResidu de lavage d=acide par la diphoterine lot No. D 500403.@ [ADiphoterine7 acid lavage residue lot No. D 500403].

A fact sheet furnished by Society Prevor is provided in Appendix 1.

The pH of the product as listed on the fact sheet is 5.84.

1.2 REACTIVE SYSTEM

1.2.1 Animals

The 6 animals used for this study were male New Zealand white rabbits furnished by Cunicole de Val de Selle, 80160 Prouzel, France.

On their arrival at C.I.T., the animals were acclimated in the experimental location for at least 5 days, during which they were observed daily. Systematic prophylactic treatment was carried out to avoid the risk of coccidial infection by adding a solution of Mucoxid7 (Véda-Cogla, 45140 Saint Jean de la Ruelle, France) to the drinking water at a dose of 137.5 mg/kg/day in a volume of 10 mL/kg during the 5 day acclimation period.

On the treatment day, the animals had a mean weight of 2.4 ∀ 0.2 kg. They were individually identified by a metallic tag inserted into the pinna of the ear.

1.2.2 Environment

The study was carried out in a conventional climate controlled animal facility. The maintenance conditions during the acclimation period and throughout the entire duration of the study were as follows:

- Temperature: 20 ∀ 3 degrees C
- Relative humidity: 50 ∀ 20 % relative humidity
- Light/Dark cycle: 12 hours of light/12 hours of darkness

The temperature and relative humidity were recorded in each study chamber. Non-recycled air was filtered with isolation filters.

The animals were individually housed in polystyrene cages equipped with food and water dispensers.

1.2.3 Food and water

Food in the form of granules ALapins entretien reference 112 C@ (U.A.R., 91360 Villemoisson-sur-Orge, France) [rabbit maintenance diet] was furnished ad libitum for the total duration of the study. A certificate of analysis of the quality of this feed and its content of principal contaminants (pesticides, heavy metals, mycotoxins, others) is provided for each lot by the supplier. The formula of this feed is shown in Appendix 2.

Potable water filtered through a 0.22 micron membrane (Société Millipore, 78140 Velizy, France) was furnished ad libitum throughout the study and distributed through water dispensers. Bacteriological and chemical analyses for principal contaminants [as above] were done regularly (Laboratoire Municipal et Regional de Rouen, 76000, Rouen, France) [Municipal and Regional Laboratory of Rouen, 76000, Rouen, France].

To the knowledge of the Study Director, there was no significant information which indicated the presence of any non-nutritive substances in the food or water at levels which could have interfered with the test product.

1.3 TREATMENT

1.3.1 Animal preparation

The day before treatment, the animal=s eyes were observed to ensure that there were no ocular injuries.

1.3.2 Product administration

A single instillation of 0.1 mL of the product was done into the conjunctival cul-de-sac of the left eye in 6 animals after having gently spread the lower eyelid from the ocular globe. The lower and upper eyelids were maintained in contact for several seconds to avoid loss of the substance. No product was instilled into the right eyes which served as controls.

The animals were placed in a plastic restraining box for 1 hour, and then were replaced into individual cages.

The eyes were not rinsed after product instillation.

1.3.3 Treatment date

The single administration was done on 06/19/90 (Day 1). The animals were observed until 06/26/90 (Day 8).

1.4 DESCRIPTION AND EVALUATION OF THE OCULAR REACTIONS

Ocular reactions were evaluated 1 hour, 24 hours, 48 hours, 72 hours, 96 hours, and 7 days after instillation of the product as the mean of the following numerical scale (J.O.R.F., October 24, 1984) [Official Journal of the French Republic, October 24, 1984]:

1.4.1 Conjunctival lesions and lacrimation

Chemosis (edema)
- No swelling.....0
- Mild swelling of the nictating membrane.....1
- Pronounced swelling with eversion of the eyelid.....2
- Swelling with the eyelids half closed.....…3
- Swelling with the eyelids closed more than half or completely.....4

with AA@ being the value obtained.

Lacrimation
- Absence of lacrimation.....0
- Mild lacrimation (excluding mild secretions which normally are present in the internal angle.....1
- Lacrimation with wetness of the eyelids and eyelashes.....2
- Lacrimation with wetness of the eyelids and eyelashes and a large area around the eye.....3

with AB@ being the value obtained.

Conjunctival erythema (redness)
- Normal vessels.....0
- Vessels clearly more injected than normal.....1
- More diffuse and bright red color, difficult to distinguish individual vessels.....2
- Diffuse deep red color, difficult to distinguish individual vessels.....3

with AC@ being the value obtained.

1.4.2 Iris lesions

Iris

- No abnormality.....0
- Iris clearly more contracted than normal; congestion; swollen; iris reacts to light but slowly (one or more of these characteristics): iris reacts to light with reflex contraction of the pupil (a slow reaction is a positive reaction).....1
- No reaction to light, hemorrhages, significant destruction (one or more or all of these characteristics).....2

with AD@ being the value obtained,

1.4.3 Corneal lesions

Cornea (direct examination and ultraviolet light [Wood=s lamp] examination)

To verify the presence or absence of corneal opacification and to evaluate surface injury, the eye should be irrigated with one or two drops of an aqueous 0.5% fluoresceine solution (but not before 24 hours).

In questionable cases regarding the presence of corneal opacity, the eye is examined using U.V. light (the injured corneal layers are distinguished by a very distinct fluorescence). Only the layer with the highest degree of opacification is chosen for the reading of the extent of opacity.

Extent of opacity

- No visible opacity, nor loss of glistening or of shine.....0
- Presence of translucent areas (diffuse or disseminated), iris details clearly visible.....…1
- Presence of an easily identifiable translucent area; iris details slightly obscured.....2
- Presence of an opalescent area, no iris details visible; pupil outline difficult to discern.....3
- Presence of total corneal opacity, rendering the iris and the pupil invisible.....4

with AE@ being the value obtained.

Surface area of Opacity

- One-fourth (or less but surface not uninvolved).....1
- Between one-fourth and one-half.....…2
- Between one-half and three-fourths.....3
- Between three-fourths and total surface.....4

with AF@ being the value obtained.

All other observed lesions are recorded.

1.5 CALCULATIONS AND INTERPRETATION OF OCULAR IRRITATION INDICES

1.5.1 Calculation methods

1.5.1.1. Index of individual ocular irritation (I.O.I.)

For each animal and each observation period, the following calculations were done:

Conjunctiva:	X = 2 (A+B+C)	(X </= 20)
Iris:	Y = 5 x D	(Y </= 10)
Cornea:	Z = 5 x E x F	(Z </= 80)

From these values, and index of **individual ocular irritation** (abbreviated as **I.O.I**.) corresponding to the sum of all values obtained (X+Y+Z) was determined for each rabbit and for each observation period.

1.5.1.2. Index of mean irritation per site

For each observation period, an index of mean ocular irritation per site (conjunctiva, iris, cornea) was calculated according to the following expressions:

Conjunctive:	sum of X / 6
Iris:	sum of Y / 6
Cornea:	sum of Z / 6

1.5.1.3. Index of mean ocular irritation (I.O.)

For each observation period, an index of **mean ocular irritation** (abbreviated as **I.O.**), corresponding to the mean of the individual ocular irritation indices of treated animals alive at the end of the study period was calculated.

1.5.1.4. Index of maximum ocular irritation (I.O. Ma.)

The greatest mean index of ocular irritation (I.O.), obtained at the various observation periods, corresponds to the **maximum ocular irritation (I.O. Ma.)** index.

1.5.2 Interpretation of the results

The maximum ocular irritation index (I.O.Ma.) allows pre-classification of the product between the classes **Anon-irritating@** and **Aextremely irritating@**; this classification must be modified if the injury is not sufficiently reversible. The reversibility of the observed lesions is evaluated by the more or less rapid and significant decrease of the I.O.; the various I.O.I. values may be used to modify this evaluation.

The interpretation of the results is carried out according to the following table.

Values of Different Indices

I.O. Ma.	I.O.*	I.O.I*	Conclusion: The Product is considered as:
< 5	= 0 after 24 hours		non-irritating
>/= 5 and <15	</= 2 after 48 hours		very mildly irritating
>/= 15 and <25	</= 2 after 96 hours		mildly irritating
>/=25 and < 50	</= 20 on day 8	on day 8: </= 30 in 6 of 6 rabbits </= 15 in at least 4/6 rabbits	irritating
>/= 50 and <80	</= 40 on day 8	on day 8: </= 60 in 6 of 6 rabbits and </=30 in at least 4 of 6 rabbits	severely irritating
>/= 80			extremely irritating

*If the conclusion stipulated by this index is not filled in, the conclusion is that of the next highest class.

1.6 ARCHIVES

The following documents:

_ the protocol and any amendments,
_ all original data,
_ correspondence,
_ the final study report and any amendments,

are retained in the archives of C.I.T. at Miserey, 27005 Evreux, France for 5 years after the conclusion of in vivo studies. At the end of this period, the archives of the study are, at the discretion of the laboratory authority, either transferred to another location or destroyed following agreement of the laboratory.

2. RESULTS (Tables 1 through 6)

No ocular reaction was observed in the animals.

Study No. 6463 TAL

Table : 1

	Scores	Day 1 (1 hour)					
	Rabbit No:	01	02	03	04	05	06
CON-JUNCTIVA (X)	Chemosis (A)	0	0	0	0	0	0
	Lacrimation (B)	0	0	0	0	0	0
	Conjunctival Erythema (C)	0	0	0	0	0	0
	(A+B+C) x 2	0	0	0	0	0	0
IRIS (Y)	Extent of Congestion (D)	0	0	0	0	0	0
	D x 5	0	0	0	0	0	0
CORNEA (Z)	Extent of Opacity (E)	0	0	0	0	0	0
	Opacity Surface area (F)	0	0	0	0	0	0
	E x F x 5	0	0	0	0	0	0
Fluoresceine		/	/	/	/	/	/
	I. O. I.	0	0	0	0	0	0
	Index of mean irritation per site		X		Y		Z
			0, 0		0, 0		0, 0
	I. O. M.			0, 0			

I. O. Ma. = 0, 0

/ = fluoresceine not used

Study no. 6463 TAL

Table : 2

Scores		Day 2 (24 hours)					
	Rabbit No:	01	02	03	04	05	06
CON-JUNCTIVA (X)	Chemosis (A)	0	0	0	0	0	0
	Lacrimation (B)	0	0	0	0	0	0
	Conjunctival Erythema (C)	0	0	0	0	0	0
	(A+B+C) x 2	0	0	0	0	0	0
IRIS (Y)	Extent of Congestion (D)	0	0	0	0	0	0
	D x 5	0	0	0	0	0	0
CORNEA (Z)	Extent of Opacity (E)	0	0	0	0	0	0
	Opacity Surface area (F)	0	0	0	0	0	0
	E x F x 5	0	0	0	0	0	0
Fluoresceine		U	U	U	U	U	U
	I. O. I.	0	0	0	0	0	0

Index of mean irritation per site	X	Y	Z
	0, 0	0, 0	0, 0

I. O. M. 0, 0

U = Fluoresceine lot No. 6574 utilized

Study No. 6463 TAL

Table : 3

Scores		Day 3 (48 hours)					
	Rabbit No:	01	02	03	04	05	06
CON-JUNCTIVA (X)	Chemosis (A)	0	0	0	0	0	0
	Lacrimation (B)	0	0	0	0	0	0
	Conjunctival Erythema (C)	0	0	0	0	0	0
	(A+B+C) x 2	0	0	0	0	0	0
IRIS (Y)	Extent of Congestion (D)	0	0	0	0	0	0
	D x 5	0	0	0	0	0	0
CORNEA (Z)	Extent of Opacity (E)	0	0	0	0	0	0
	Opacity Surface area (F)	0	0	0	0	0	0
	E x F x 5	0	0	0	0	0	0
Fluoresceine		/	/	/	/	/	/
	I. O. I.	0	0	0	0	0	0
	Index of mean irritation per site		X		Y		Z
			0, 0		0, 0		0, 0
	I. O. M.			0, 0			

/ = Fluoresceine not used

Study No. 6463 TAL

Table : 4

Scores		Day 4 (72 hours)					
	Rabbit No:	01	02	03	04	05	06
CON-JUNCTIVA (X)	Chemosis (A)	0	0	0	0	0	0
	Lacrimation (B)	0	0	0	0	0	0
	Conjunctival Erythema (C)	0	0	0	0	0	0
	(A+B+C) x 2	0	0	0	0	0	0
IRIS (Y)	Extent of Congestion (D)	0	0	0	0	0	0
	D x 5	0	0	0	0	0	0
CORNEA (Z)	Extent of Opacity (E)	0	0	0	0	0	0
	Opacity Surface area (F)	0	0	0	0	0	0
	E x F x 5	0	0	0	0	0	0
Fluoresceine		/	/	/	/	/	/
	I. O. I.	0	0	0	0	0	0
	Index of mean irritation per site		X		Y		Z
			0, 0		0, 0		0, 0
	I. O. M.			0, 0			

/ = Fluoresceine not used

Study No. 6463 TAL

Table : 5

Scores		Day 5					
	Rabbit No:	01	02	03	04	05	06
CON-JUNCTIVA (X)	Chemosis (A)	0	0	0	0	0	0
	Lacrimation (B)	0	0	0	0	0	0
	Conjunctival Erythema (C)	0	0	0	0	0	0
	(A+B+C) x 2	0	0	0	0	0	0
IRIS (Y)	Extent of Congestion (D)	0	0	0	0	0	0
	D x 5	0	0	0	0	0	0
CORNEA (Z)	Extent of Opacity (E)	0	0	0	0	0	0
	Opacity Surface area (F)	0	0	0	0	0	0
	E x F x 5	0	0	0	0	0	0
Fluoresceine		/	/	/	/	/	/
	I. O. I.	0	0	0	0	0	0
	Index of mean irritation per site		X		Y		Z
			0, 0		0, 0		0, 0
	I. O. M.			0, 0			

/ = Fluoresceine not used

Study No. 6463 TAL

Table : 6

Scores		Day 8					
	Rabbit No:	01	02	03	04	05	06
CON-JUNCTIVA (X)	Chemosis (A)	0	0	0	0	0	0
	Lacrimation (B)	0	0	0	0	0	0
	Conjunctival Erythema (C)	0	0	0	0	0	0
	(A+B+C) x 2	0	0	0	0	0	0
IRIS (Y)	Extent of Congestion (D)	0	0	0	0	0	0
	D x 5	0	0	0	0	0	0
CORNEA (Z)	Extent of Opacity (E)	0	0	0	0	0	0
	Opacity Surface area (F)	0	0	0	0	0	0
	E x F x 5	0	0	0	0	0	0
Fluoresceine		/	/	/	/	/	/
	I. O. I.	0	0	0	0	0	0
	Index of mean irritation per site		X		Y		Z
			0, 0		0, 0		0, 0
	I. O. M.			0, 0			

/ = Fluoresceine not used

Appendices

Study No. 6463 TAL

Fact Sheet on the Tested Product

Fact Sheet on the Tested Product

Name of Laboratory: PREVOR

Name: Diphoterine acid lavage residue

Lot Number: D 500403

Physical Characteristics:
- liquid
- color: colorless
- pH: 5.84
- density: about 1
- other known characteristics: non-caustic aqueous solution

Vehicle to utilize (if necessary for the study):
- preferred vehicle:
- vehicle in which the product is soluble:

Precautions to observe: none

Storage conditions:

Measures to take in case of contamination: washing

Elimination of the remainder:

- return: no
- destruction: method

SIC PREVOR
Moulin de Verville
95760 VALMONDOIS
(FRANCE) Telephone (3) 4731313

Date: 14 March 90
Confirmatory Signature: [signature]

To aid us in following Good Laboratory Practices, the packaging of received products should be labeled with the name of the laboratory and the name and lot number of the product.
It is preferable to also precisely list the quantity of the product: 250 ml.

2. Dietary Formula

Study No. 6463 TAL

COMPLETE DIET FOR
RABBIT MAINTENANCE
In the form of 4.5 mm granules
Packaging: 25 Kg sack

Daily ration: 100 to 150 gram rabbits, according to the breed and weight, water ad libitum

FORMULA %

Cereals, sugar 43.8
Vegetable materials 49
Vegetable protein (oil) 4.2
Mineral and vitamin content 3

ANALYSIS MEAN %

Caloric value (in cal/Kg) 2,200
Water 10
Peptides 13
Lipids 2.7
Sugar (E.N.A.) 49.3
Cellulose (Weende) 17
Minerals 8

AMINO ACIDS
(calculated in mg/Kg)

Arginine 6,800
Cystine 2,100
Lysine 4,600
Methionine 1,600
Tryptophane 1,400
Glycine 5,200

Available in AControl Reference@ quality: 112 C

7, rue Gallieni: VILLEMOISSON 91360
EPINAY/ORGE Telephone 69.04.03.57
Telex: UAR 691716F

MINERALS
(calculated in mg/Kg)

	Mean Natural Content	Added Content	Total
P	3,500	3,500	7,000
Ca	4,500	4,500	9,000
K	11,600	0	11,600
Na	400	1,600	2,000
Mg	2,100	100	2,200
Mn	40	40	80
Fe	160	140	300
Cu	12	15	27
Zn	30	45	75
Co	0.1	1.5	1.6

VITAMINES
(calculated per Kg)

	Mean Natural Content	Added Content	Total
Vitamin A	2,850 IU	6,500 IU	9,350 IU
Vitamin D_3	30 IU	1,000 IU	1,030 IU
Vitamin B_1	4.30 mg	0 mg	4.30 mg
Vitamin B_2	3.80 mg	0 mg	3.80 mg
Vitamin B_3	16 mg	0 mg	16 mg
Vitamin B_6	1 mg	1 mg	2 mg
Vitamin E	16 mg	10 mg	26 mg
Vitamin K_3	6 mg	1 mg	7 mg
Vitamin PP	55 mg	5 mg	60 mg
Choline	850 mg	200 mg	1,050 mg

Available in AControl Reference@ quality: 112 C

7, rue Gallieni: VILLEMOISSON 91360
EPINAY/ORGE Telephone 69.04.03.57
Telex: UAR 691716F

Study number 6462 TAL CONFIDENTIAL

DIPHOTERINE SODIUM HYDROXIDE LAVAGE RESIDUE EVALUATION OF OCULAR IRRITATION IN THE RABBIT

Address

Society Prevor
Madame D. Jahan
Moulin de Verville
95760 Valmontois [sic]
France

Date: 07/10/90

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[*Translator=s note: page numbers refer to the original French document]

CERTIFICATION OF THE STUDY DIRECTOR

This study has been carried out in conformity with the protocol established with Society Prevor, agreement of September 21, 1984 published in the Journal Officiel de la Républic Français (J.O.R.F. of October 24, 1984 [sic]) [Official Journal of the French Republic] and according to the Good Laboratory Practice rules (France, Ministère de la Santé, May 31, 1983) [France, Ministry of Health].

I certify that this report is a true and accurate description of the procedures utilized and the results obtained while conducting this study.

This study was done at the Centre International de Toxicologie (C.I.T.) [International Toxicology Center] at Miserey, 27005 Evereux, France.

Toxicology

[Signature]

J. Clouzeau Date: 07/10/90
Biologist
Head of the Local Tolerance
and Acute Toxicity Service

OTHER SCIENTIFIC PARTICIPANTS IN THIS STUDY

[Signature]

Pharmacy
Product Preparation

M.H. Read Date: 07/10/90
Pharmacist Biologist

Study No. 6463 TAL

ACCOUNT OF THE QUALITY ASSURANCE UNIT

The protocol and the report were inspected by the Quality Assurance Unit of the International Toxicology Center on the following dates:

Inspection	Inspection Date	Date of Inspection Report
Protocol	05/16/90	05/16/90
Report (1st Draft)	07/05/90	07/06/90
Report (final)	07/10/90	07/10/90

Studies (of the same type) were routinely inspected on the following dates:

Treatment/Product	06/05/90	06/05/90
Data Recording	06/05/90	06/05/90
Records	06/22/90	06/22/90
Product/Preparation	06/11/90	06/11/90

Inspections were carried out in accordance with the standard operating procedures of C.I.T. and Good Laboratory Practices (France, Ministère de la Santé, May 31, 1983) [France, Ministry of Health].

[Signature]

M. Labiche Date: 07/10/90
Head of the Quality
Assurance Unit and
Scientific Archives

Study No. 6463 TAL

SUMMARY

At the request of Society Prevor, ocular irritation which might be produced by the product **DIPHOTERINE7 SODIUM HYDROXIDE LAVAGE RESIDUE** was evaluated in the rabbit in conformity with the agreement of September 21, 1984 published in the Journal Officiel de la Républic Française [Official Journal of the French Republic] and according to Good Laboratory Practice rules (France, Ministère de la Santé, May 31, 1983) [France, Ministry of Health].

Materials and Methods

A single dose of 0.1 mL of the product was placed in the conjunctival sac of the left eye of 6 New Zealand white rabbits. No eye rinsing was done after application of the product. The ocular reaction was observed 1 hour after application, and then on days 2, 3, 4, 5, and 8.

Results

No ocular reaction was observed in these animals.

Conclusion

Under our experimental conditions and using the rating scale developed by Kay and Calandra, the product **DIPHOTERINE7 SODIUM HYDROXIDE LAVAGE RESIDUE** is considered to be **non-irritating** by the ocular route in rabbits.

1. MATERIALS AND METHODS

1.1 PRODUCT

The product **DIPHOTERINE7 SODIUM HYDROXIDE LAVAGE RESIDUE** furnished by Society Prevor, Moulin de Verville, 95760 Valmontois [sic], France was in the form of a colorless liquid.

The product was packaged in one glass bottle and was received at C.I.T. on 05/21/90 with the label AResidu de lavage de soude par la diphotérine lot No. D 500403.@ [ADiphoterine7 sodium hydroxide lavage residues lot No. D 500403].

A fact sheet furnished by Society Prevor is provided in Appendix 1.

The pH of the product as listed on the fact sheet is 8.82.

1.2 REACTIVE SYSTEM

1.2.1 Animals

The 6 animals used for this study were male New Zealand white rabbits furnished by Cunicole breeders de Val de Selle, 80160 Prouzel, France.

On their arrival at C.I.T., the animals were acclimated in the experimental location for at least 5 days, during which they were observed daily. Systematic prophylactic treatment was carried out to avoid the risk of coccidial infection by adding a solution of Mucoxid7 (Véda-Cogla, 45140 Saint Jean de la Ruelle, France) to the drinking water at a dose of 137.5 mg/kg/day in a volume of 10 mL/kg during the 5 day acclimation period.

On the treatment day, the animals had a mean weight of 3.0 ∀ 0.1 Kg. They were individually identified by a metallic tag inserted into the pinna of the ear.

1.2.2 Environment

The study was carried out in a conventional climate controlled animal facility. The maintenance conditions during the acclimation period and throughout the entire duration of the study were as follows:

- Temperature: 20 ∀ 3 degrees C
- Relative humidity: 50 ∀ 20 %
- Light/Dark cycle: 12 hours of light/12 hours of darkness

The temperature and relative humidity were recorded in each study chamber. Non-recycled air was filtered with isolation filters.

The animals were individually housed in polystyrene cages equipped with food and water dispensers.

1.2.3 Food and water

Food in the form of granules ALapins entretien reference 112 C@ (U.A.R., 91360 Villemoisson-sur-Orge, France [rabbit maintenance diet; see Appendix 2, Dietary Formula] was furnished ad libitum for the total duration of the study. A certificate on analysis of the quality of this feed and its content of principal contaminants (pesticides, heavy metals, mycotoxins, others) is provided for each lot by the supplier. The formula of this feed is shown in Appendix 2.

Potable water filtered through a 0.22 micron membrane (Société Millipore, 78140 Velizy, France) was furnished ad libitum throughout the study and distributed through water dispensers. Bacteriological and chemical analyses for principal contaminants [as above] were done regularly (Laboratoire Municipal et Regional de Rouen, 76000, Rouen, France) [Municipal and Regional Laboratory of Rouen, 76000, Rouen, France].

To the knowledge of the Study Director, there was no significant information which indicated the present of any non-nutritious substances in the food or water at levels which could have interfered with the test product.

1.3 TREATMENT

1.3.1 Animal preparation

The day before treatment, the animal=s eyes were observed to ensure that there were no ocular injuries.

1.3.2 Product Administration

A single instillation of 0.1 mL of the product was done into the conjunctival cul-de-sac of the left eye in 6 animals after having gently spread the lower eyelid from the ocular globe. The lower and upper eyelids were maintained in contact for several seconds to avoid loss of the substance. No product was instilled into the right eyes which served as controls.

The animals were placed in a plastic restraining box for 1 hour, and then were replaced into individual cages.

The eyes were not rinsed after product instillation.

1.3.3 Treatment date

The single administration was done on 06/19/90 (Day 1). The animals were observed until 06/26/90 (Day 8).

1.4. DESCRIPTION AND EVALUATION OF THE OCULAR REACTIONS

Ocular reactions were evaluated 1 hour, 24 hours, 48 hours, 72 hours, 96 hours, and 7 days after instillation of the product as the mean of the following numerical scale (J.O.R.F., October 24, 1984) [Official Journal of the French Republic, October 24, 1984]:

1.4.1 Conjunctival lesions and lacrimation

Chemosis (edema)
- No swelling.....0
- Mild swelling of the nictating membrane.....1
- Pronounced swelling with eversion of the eyelid.....2
- Swelling with the eyelids half closed.....…3
- Swelling with the eyelids closed more than half or completely.....4

with AA@ being the value obtained.

Lacrimation
- Absence of lacrimation.....0
- Mild lacrimation (excluding mild secretions which normally are present in the internal angle.....…1
- Lacrimation with wetness of the eyelids and eyelashes.....…..2
- Lacrimation with wetness of the eyelids and eyelashes and a large area around the eye.....3

with AB@ being the value obtained.

Conjunctival erythema (redness)
- Normal vessels.....0
- Vessels clearly more injected than normal.....1
- More diffuse and bright red color, difficult to distinguish individual vessels.....2
- Diffuse deep red color, difficult to distinguish individual vessels.....3

with AC@ being the value obtained.

1.4.2 Iris lesions

Iris

- No abnormality.....0
- Iris clearly more contracted than normal; congestion; swollen; iris reacts to light but slowly (one or more of these characteristics): iris reacts to light with reflex contraction of the pupil (a slow reaction is a positive reaction).....1
- No light reaction, hemorrhages, significant destruction (one or more or all of these characteristics).....2

with AD@ being the value obtained,

1.4.3 Corneal lesions

Cornea (direct examination and ultraviolet light [Wood=s lamp] examination)

To verify the presence or absence of corneal opacification and to evaluate surface injury, the eye should be irrigated with one or two drops of an aqueous 0.5% fluoresceine solution (but not before 24 hours).

In questionable cases regarding the presence of corneal opacity, the eye is examined using U.V. light (the injured corneal areas are distinguished by a very distinct fluorescence). Only the area with the highest degree of opacification is chosen for the reading of the extent of opacity.

Extent of opacity

- No visible opacity, nor loss of glistening or of shine.....0
- Presence of translucent areas (diffuse or disseminated), iris details clearly visible.....1
- Presence of an easily identifiable translucent area; iris details slightly obscured.....2
- Presence of an opalescent area, no iris details visible; pupil outline difficult to discern.....3
- Presence of total corneal opacity, rendering the iris and the pupil invisible.....4

with AE@ being the value obtained.

Surface area of Opacity

- One-fourth (or less but surface not uninjured).....1
- Between one-fourth and one-half.....…2
- Between one-half and three-fourths.....3
- Between three-fourths and total surface.....…4

with AF@ being the value obtained.

All other observed lesions are recorded.

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1.5 CALCULATIONS AND INTERPRETATION OF OCULAR IRRITATION INDICES

1.5.1 Calculation methods

1.5.1.1 Index of individual ocular irritation (I.O.I.)

For each animal and each observation period, the following calculations were done:

Conjunctiva:	X = 2 (A+B+C)	(X </= 20)
Iris:	Y = 5 x D	(Y </= 10)
Cornea:	Z = 5 x E x F	(Z </= 80)

From these values, and index of **individual ocular irritation** (abbreviated as **I.O.I**.) corresponding to the sum of all values obtained (X+Y+Z) was determined for each rabbit and for each observation period.

1.5.1.2 Index of mean irritation per site

For each observation period, an index of mean ocular irritation per site (conjunctiva, iris, cornea) was calculated according to the following expressions:

Conjunctiva:	sum of X / 6
Iris:	sum of Y / 6
Cornea:	sum of Z / 6

1.5.1.3 Index of mean ocular irritation (I.O.)

For each observation period, an index of **ocular irritation** (abbreviated as **I.O.**), corresponding to the mean of the individual ocular irritation indices of treated animals alive at the end of the study period, was calculated.

1.5.1.4 Index of maximum ocular irritation (I.O. Ma.)

The greatest mean index of ocular irritation (I.O.) obtained at the various observation periods corresponds to the **maximum ocular irritation (I.O. Ma.)** index.

1.5.2 Interpretation of the results

The maximum ocular irritation index (I.O.Ma.) allows pre-classification of the product between the classes **Anon-irritating@** and **Aextremely irritating@**; this classification must be modified of the injury is not sufficiently reversible. The reversibility of the observed lesions is evaluated by the more or less rapid and significant decrease of the I.O.; the various I.O. values may be used to modify this evaluation.

The interpretation of the results is carried out according to the following table.

Values of Different Indices

I.O. Ma.	I.O.*	I.O.I*	Conclusion: The Product is considered as:
< 5	= 0 after 24 hours		non-irritating
>/= 5 and <15	</= 2 after 48 hours		very mildly irritating
>/= 15 and <25	</= 2 after 96 hours		mildly irritating
>/=25 and < 50	</= 20 on day 8	on day 8: </= 30 in 6 of 6 rabbits </= 15 in at least 4/6 rabbits	irritating
>/= 50 and <80	</= 40 on day 8	on day 8: </= 60 in 6 of 6 rabbits and </=30 in at least 4 of 6 rabbits	severely irritating
>/= 80			extremely irritating

*If the conclusion stipulated by this index is not filled in, the conclusion is that of the next highest class.

1.6 ARCHIVES

The following documents:

_ the protocol and any amendments,
_ all original data,
_ correspondence,
_ the final study report and any amendments,

are retained in the archives of C.I.T. at Miserey, 27005 Evreux, France for 5 years after the conclusion of in vivo studies. At the end of this period, the archives of the study are, at the discretion of the laboratory authority, either transferred to another location or destroyed following agreement of the laboratory.

2.0 RESULTS (Tables 1 through 6)

No ocular reaction was observed in the animals.

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Table : 1

Scores		Day 1 (1 hour)					
	Rabbit No:	01	02	03	04	05	06
CON-JUNCTIVA (X)	Chemosis (A)	0	0	0	0	0	0
	Lacrimation (B)	0	0	0	0	0	0
	Conjunctival Erythema (C)	0	0	0	0	0	0
	(A+B+C) x 2	0	0	0	0	0	0
IRIS (Y)	Extent of Congestion (D)	0	0	0	0	0	0
	D x 5	0	0	0	0	0	0
CORNEA (Z)	Extent of Opacity (E)	0	0	0	0	0	0
	Opacity Surface area (F)	0	0	0	0	0	0
	E x F x 5	0	0	0	0	0	0
Fluoresceine		/	/	/	/	/	/
	I. O. I.	0	0	0	0	0	0
	Index of mean irritation per site		X		Y		Z
			0, 0		0, 0		0, 0
	I. O. M.			0, 0			

I. O. Ma. = 0, 0

/ = fluoresceine not used

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Table : 2

Scores		Day 2 (24 hours)					
	Rabbit No:	01	02	03	04	05	06
CON-JUNCTIVA (X)	Chemosis (A)	0	0	0	0	0	0
	Lacrimation (B)	0	0	0	0	0	0
	Conjunctival Erythema (C)	0	0	0	0	0	0
	(A+B+C) x 2	0	0	0	0	0	0
IRIS (Y)	Extent of Congestion (D)	0	0	0	0	0	0
	D x 5	0	0	0	0	0	0
CORNEA (Z)	Extent of Opacity (E)	0	0	0	0	0	0
	Opacity Surface area (F)	0	0	0	0	0	0
	E x F x 5	0	0	0	0	0	0
Fluoresceine		U	U	U	U	U	U
	I. O. I.	0	0	0	0	0	0
	Index of mean irritation per site		X		Y		Z
			0, 0		0, 0		0, 0
	I. O. M.			0, 0			

U = Fluoresceine lot No. 6574 utilized

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Table : 3

	Scores	Day 3 (48 hours)					
	Rabbit No:	01	02	03	04	05	06
CON-JUNCTIVA (X)	Chemosis (A)	0	0	0	0	0	0
	Lacrimation (B)	0	0	0	0	0	0
	Conjunctival Erythema (C)	0	0	0	0	0	0
	(A+B+C) x 2	0	0	0	0	0	0
IRIS (Y)	Extent of Congestion (D)	0	0	0	0	0	0
	D x 5	0	0	0	0	0	0
CORNEA (Z)	Extent of Opacity (E)	0	0	0	0	0	0
	Opacity Surface area (F)	0	0	0	0	0	0
	E x F x 5	0	0	0	0	0	0
Fluoresceine		/	/	/	/	/	/
	I. O. I.	0	0	0	0	0	0

Index of mean irritation per site	X	Y	Z
	0, 0	0, 0	0, 0

I. O. M. 0, 0

/ = Fluoresceine not used

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Table : 4

Scores		Day 4 (72 hours)					
Rabbit No:		01	02	03	04	05	06
CON-JUNCTIVA (X)	Chemosis (A)	0	0	0	0	0	0
	Lacrimation (B)	0	0	0	0	0	0
	Conjunctival Erythema (C)	0	0	0	0	0	0
	(A+B+C) x 2	0	0	0	0	0	0
IRIS (Y)	Extent of Congestion (D)	0	0	0	0	0	0
	D x 5	0	0	0	0	0	0
CORNEA (Z)	Extent of Opacity (E)	0	0	0	0	0	0
	Opacity Surface area (F)	0	0	0	0	0	0
	E x F x 5	0	0	0	0	0	0
Fluoresceine		/	/	/	/	/	/
I. O. I.		0	0	0	0	0	0

Index of mean irritation per site	X	Y	Z
	0, 0	0, 0	0, 0

I. O. M. 0, 0

/ = Fluoresceine not used

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Table : 5

Scores		Day 5					
	Rabbit No:	01	02	03	04	05	06
CON-JUNCTIVA (X)	Chemosis (A)	0	0	0	0	0	0
	Lacrimation (B)	0	0	0	0	0	0
	Conjunctival Erythema (C)	0	0	0	0	0	0
	(A+B+C) x 2	0	0	0	0	0	0
IRIS (Y)	Extent of Congestion (D)	0	0	0	0	0	0
	D x 5	0	0	0	0	0	0
CORNEA (Z)	Extent of Opacity (E)	0	0	0	0	0	0
	Opacity Surface area (F)	0	0	0	0	0	0
	E x F x 5	0	0	0	0	0	0
Fluoresceine		/	/	/	/	/	/
	I. O. I.	0	0	0	0	0	0
	Index of mean irritation per site		X		Y		Z
			0, 0		0, 0		0, 0
	I. O. M.			0, 0			

/ = Fluoresceine not used

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Table : 6

	Scores	Day 8					
	Rabbit No:	01	02	03	04	05	06
CON-JUNCTIVA (X)	Chemosis (A)	0	0	0	0	0	0
	Lacrimation (B)	0	0	0	0	0	0
	Conjunctival Erythema (C)	0	0	0	0	0	0
	(A+B+C) x 2	0	0	0	0	0	0
IRIS (Y)	Extent of Congestion (D)	0	0	0	0	0	0
	D x 5	0	0	0	0	0	0
CORNEA (Z)	Extent of Opacity (E)	0	0	0	0	0	0
	Opacity Surface area (F)	0	0	0	0	0	0
	E x F x 5	0	0	0	0	0	0
Fluoresceine		/	/	/	/	/	/
	I. O. I.	0	0	0	0	0	0

Index of mean irritation per site	X	Y	Z
	0, 0	0, 0	0, 0

I. O. M. 0, 0

/ = Fluoresceine not used

Appendices

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Fact Sheet on the Tested Product

Fact Sheet on the Tested Product

Name of Laboratory: PREVOR

Name: Diphoterine sodium hydroxide lavage residue

Lot Number: D 500403

Physical Characteristics:
liquid
color: colorless
pH: 8.82
density: about 1
other known characteristics: non-caustic aqueous solution

Vehicle to utilize (if necessary for the study):
preferred vehicle:
vehicle in which the product is soluble:

Precautions to observe: none

Storage conditions:

Measures to take in case of contamination: washing

Elimination of the remainder:

return: no
destruction: method

SIC PREVOR
Moulin de Verville
95760 VALMONDOIS
(FRANCE) Telephone (3) 4731313

Date: 14 March 90
Confirmatory Signature: [signature]

To aid us in following Good Laboratory Practices, the packaging of received products should be labeled with the name of the laboratory and the name and lot number of the product.
It is preferable to also precisely list the quantity of the product: 250 ml.

2. Dietary Formula

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COMPLETE DIET FOR
RABBIT MAINTENANCE
In the form of 4.5 mm granules
Packaging: 25 Kg sack

Daily ration: 100 to 150 gram rabbits, according to the breed and weight, water ad libitum

FORMULA %

Cereals, sugar 43.8
Vegetable materials 49
Vegetable protein (oil) 4.2
Mineral and vitamin content 3

ANALYSIS MEAN %

Caloric value (in cal/Kg) 2,200
Water 10
Peptides 13
Lipids 2.7
Sugar (E.N.A.) 49.3
Cellulose (Weende) 17
Minerals 8

AMINO ACIDS
(calculated in mg/Kg)

Arginine 6,800
Cystine 2,100
Lysine 4,600
Methionine 1,600
Tryptophane 1,400
Glycine 5,200

Available in AControl Reference@ quality: 112 C

7, rue Gallieni: VILLEMOISSON 91360
EPINAY/ORGE Telephone 69.04.03.57
Telex: UAR 691716F

MINERALS
(calculated in mg/Kg)

	Mean Natural Content	Added Content	Total
P	3,500	3,500	7,000
Ca	4,500	4,500	9,000
K	11,600	0	11,600
Na	400	1,600	2,000
Mg	2,100	100	2,200
Mn	40	40	80
Fe	160	140	300
Cu	12	15	27
Zn	30	45	75
Co	0.1	1.5	1.6

VITAMINES
(calculated per Kg)

	Mean Natural Content	Added Content	Total
Vitamin A	2,850 IU	6,500 IU	9,350 IU
Vitamin D_3	30 IU	1,000 IU	1,030 IU
Vitamin B_1	4.30 mg	0 mg	4.30 mg
Vitamin B_2	3.80 mg	0 mg	3.80 mg
Vitamin B_3	16 mg	0 mg	16 mg
Vitamin B_6	1 mg	1 mg	2 mg
Vitamin E	16 mg	10 mg	26 mg
Vitamin K_3	6 mg	1 mg	7 mg
Vitamin PP	55 mg	5 mg	60 mg
Choline	850 mg	200 mg	1,050 mg

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